ORIGINAL ARTICLE

ISSN 1598-1703 (Print) ISSN 2287-6782 (Online)
Korean J Endocr Surg 2016;16:36-41
http://dx.doi.org/10.16956/kaes.2016.16.2.36

The Korean Journal of Endocrine Surgery

$BRAF^{V600E}$ Test for Suspicious Lateral Lymph Nodes in Papillary Thyroid Cancer

Wan Wook Kim, Hyang Hee Choi[1], Jeeyeon Lee, Seung Ook Hwang, Ho Yong Park, Ji-Young Park[2], Jin Hyang Jung

Department of Surgery, Kyungpook National University School of Medicine, [1]Department of Surgery, Daegu Health College Hospital, [2]Department of Pathology, Kyungpook National University School of Medicine, Daegu, Korea

Purpose: This study evaluates the $BRAF^{V600E}$ test with fine-needle aspiration cytology (FNAC) for lateral lymph node (LN) metastasis in papillary thyroid carcinoma (PTC).
Methods: Ninety-one patients, including 95 lateral LNs with possible PTC metastasis, were consecutively entered into the study. We analyzed the accuracy of results for the $BRAF^{V600E}$ test and FNAC for lateral LN metastasis in PTC.
Results: Modified radical neck dissection was performed for 34 cases due to lateral LN metastasis. The sensitivity of FNAC was 88.2% , and the LN-$BRAF^{V600E}$ test 64.7%. The specificity and positive predictive value (PPV) were 100% for both tests. The negative predictive values (NPV) were FNAC, 93.8%, and LN-$BRAF^{V600E}$, 83.6%. For samples positive with either the $BRAF^{V600E}$ test or FNAC, the sensitivity was 94.1%, specificity 100%, PPV value 100%, and NPV 96.8%.
Conclusion: This study suggests that the complementary LN-$BRAF^{V600E}$ test with FNAC is a supportive diagnostic method for PTC patients with indeterminate or non-diagnostic suspicious lateral LNs.

Key Words: $BRAF^{V600E}$ mutation, Lymph node, Metastasis, Papillary thyroid cancer

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (2014R1A5A 2009242).
This study was supported by a grant from the National R&D Program for Cancer Control, Ministry of Health and Welfare, Republic of Korea (1420040).

Received May 9, 2016,
Revised May 29, 2016,
Accepted June 4, 2016
Correspondence: **Jin Hyang Jung**
Department of Surgery, Kyungpook National University School of Medicine, 807 Hoguk-ro, Buk-gu, Daegu 41404, Korea
Tel: +82-53-200-2704
Fax: +82-53-200-2027
E-mail: jjh01@knu.ac.kr

INTRODUCTION

Papillary thyroid cancer (PTC) is the most common type of thyroid cancer, comprising 80~90% of cases. The incidence of LN metastasis among patients diagnosed with PTC is approximately 30~80%.(*1,2*) Additionally, it has been reported that the incidence of local or regional recurrence after the surgery is almost 25~30%.(*3,4*) Accurate differential diagnoses for thyroid nodules and lymph node (LN) is crucial for determining the type of therapy, including extent of initial surgery and follow-up strategy. To detect LN metastasis, ultrasonography (USG) and fine needle aspiration cytology (FNAC) play a crucial role. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of FNAC for LNs are reported to be 77.3, 98.0, 98.9, 64.0, and 83.3%, respectively, if it is performed alone.(*5*) Recently, PTC has been reported to have a high incidence of $BRAF^{V600E}$ mutations in 80~90% of patients with PTC in Koreans.(*2*) Based on these reports, for suspicious thyroid nodules, FNAC is performed together with the $BRAF^{V600E}$ mutation test to compensate for the weak point of FNAC.(*2,6-9*) The purpose of this study was to compare the diagnostic value of this new method with that of existing cytology, to determine whether this new approach could complement FNAC when concurrently implementing FNAC and the $BRAF^{V600E}$ mutation test for the diagnosis of lateral cervical LNs.

METHODS

In this study, a total of 91 patients, including 95 LNs, who received USG-guided FNAC and surgery at the Department of Breast & Thyroid surgery, Kyungpook National University Hospital, from January 2012 to January 2014, were consecutively entered into the study. Three patients who were transferred to other hospitals were excluded. Inclusion criteria were the suspicion of lateral neck LN metastasis from preoperative USG or the detection of ^{18}F-fluorodeoxyglucose (FDG) uptake in the lateral neck LN by positron emission tomography/computed tomography (PET-CT) in patients diagnosed with or suspected of having PTC. The loss of the LN hilum, a long/short LN ratio <2, calcification or cystic change, asymmetric cortical thickening and LNs with peripheral and scattered vascularity were considered as findings for suspicious LNs. For lateral LNs, the site (level II∼V), shape (flat, round, taller than wide, and irregular), maximum/minimum diameter, long/short ratio, echogenicity, presence of a hilum, cystic change, calcification, and vascularity were determined by the USG. For a suspicious LN, USG-guided FNAC was administered three times: two samples received FNAC (liquid-based cytology- BD SurePath®, North Ryde, Australia) and 1 sample underwent BRAF^{V600E} mutation test (real-Q BRAF detection Kit, BioSewoom Inc., Seoul, Korea). The sensitivity, specificity, PPV and NPV of the BRAF^{V600E} test were compared with that of FNAC, and the results of combined administration of the BRAF test and FNAC were investigated to determine the best test method. At the early stage of the study, a PET/CT and thyroglobulin (Tg) test were not given. However, Tg-LN tests were implemented afterward to a total of 33 cases to compare the accuracy of these tests.

Data were analyzed using SPSS 20.0 software for Window® (SPSS Inc, Chicago, USA), categorical variables were compared using the Chi-square or Fisher exact tests, and continuous variables were analyzed using the independent t-test or Mann-Whitney test. The criterion for statistical significance was considered to be P value <0.05. The Institutional Review Board of Kyungpook National University reviewed and approved the study (knumcbio_12-1010).

RESULTS

The number of cases with LNs that was administered FNAC at levels II, III, IV and V due to suspicious metastatic LN was 4, 34, 51 and 6, respectively (Total 95 cases). The mean maximum size of LNs that underwent FNAC was 1.4±0.6 (range: 0.3∼3.0), the loss of echo in the LN hilum was found in all 34 cases with LN metastases (100%) and a long/short ratio ≤ 2 was found in 29 cases (85.3%). A total of 11 cases out of 17 (64.7%) with LN calcification was diagnosed with metastasis. Additionally, in 11 cases with cystic LN changes, 10 (90.9%) were diagnosed with metastasis. The average results for the Tg test for the group without LN metastasis was 1.9±2.5 ng/ml (range: 0.24∼8.3), and for the group with metastasis, it was 46,939.0±94,536.2 ng/ml (range: 0.34∼281,060.0). Of 61 cases, 67.0% had a PET/CT scan, and among them, the FDG uptake was detected in 38 cases (62.3%), and 14 cases (36.8%) were diagnosed with LN metastasis (Table 1). There were 60 cases performed BRAF^{V600E} mutation test on both thyroid nodule and LN, a total 48 cases (80.0%) were diagnosed with a BRAF^{V600E} mutation. In these 48 cases, 10 cases (20.8%) were diagnosed with a BRAF^{V600E} mutation both in the thyroid nodule and a LN, and 1 case (2.1%) was diagnosed a thyroid nodule negative and LN positive. The rest of the 37 cases (77.1%) were diagnosed as thyroid

Table 1. The diagnostic accuracy of PET/CT for lateral LN metastasis in papillary thyroid carcinoma

	Positive LN metastasis on permanent pathology after surgery (n=19)	Negative LN metastasis on tests before surgery (n=42)	P value
Positive FDG uptake on PET/CT (n=38)	14 (36.8%)	24 (63.2%)	0.925
Negative FDG uptake on PET/CT (n=23)	5 (30.4%)	18 (69.6%)	

PET/CT = positron emission tomography/computed tomography; LN = lymph node; FDG = ^{18}F-fluorodeoxyglucose.
Sensitivity: 14/19=73.7%, Specificity: 18/42=42.9%, Positive predictive value: 14/38=36.8%, Negative predictive value: 18/23=78.3%.

nodule positive and LN negative. Forty-eight cases with a $BRAF^{V600E}$ mutation were all diagnosed with PTC.

The diagrams of the patients are as follows (Fig. 1). A total of 72 patients diagnosed with cancer underwent surgery, and among them, 37 patients received thyroid surgery and central LN dissection without lateral LN metastasis. Thirty-four patients received modified radical neck dissection (mRND), and one patient was administered LN picking. The rest of the 19 PTC patients who had received thyroid operation previously and had BRAF mutation test due to suspicious finding on lateral LN, were not diagnosed with metastasis; thus, they were under observation and follow-up only. Out of 34 patients receiving mRND, 30 patients (88.2%) were diagnosed with metastasis using FNAC and 22 patients (64.7%) were diagnosed as positive via the $BRAF^{V600E}$ mutation test. The rest of 4 patients (11.8%) among 34 patients were found to be negative according to FNAC but diagnosed with metastasis though other tests. Among these 4 patients with false negative in FNAC, 2 were found to be positive according to the $BRAF^{V600E}$ mutation test; thus, they underwent a frozen section biopsy during surgery and were then diagnosed with metastasis and received mRND, and 1 patient had cystic change in the LN was found to be negative according to both FNAC and the $BRAF^{V600E}$ mutation test but received mRND due to high Tg content (41,000 ng/ml). The last patient was found to have a Tg of 0.34 ng/ml, and it was negative according to both FNAC and the $BRAF^{V600E}$ test but found to have a calcific LN, a suspicious finding, upon USG. Thus, frozen section biopsy of the LN was conducted during surgery, and because the LN was diagnosed with metastasis with the frozen biopsy, mRND was used. BRAF mutation test was helpful and informative in two patients with only BRAF mutation positive (Table 2).

The sensitivity, specificity, PPV and NPV of FNAC were

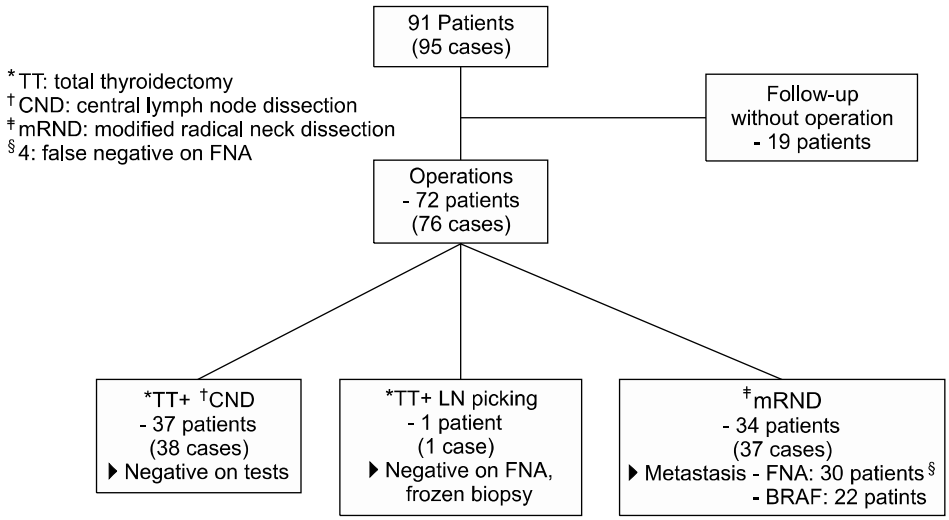

Fig. 1. The diagram of the patients who received USG-guided FNAC and surgery.

Table 2. Clinical characteristics of 4 patients with false negative FNAC on lateral LN metastasis of papillary thyroid carcinoma

Case	Age/sex	Location	Size (max)	LN-Tg (ng/ml)	LN-BRAF mutation	FDG on PET/CT	Suspicious finding on USG
1	31/F	Lt level II	2.5 cm	2.2	Positive	Uptake (−)	Hypoechoic, loss of hilum
2	47/F	Rt level III	1.5 cm	0.4	Positive	Uptake (−)	L/S ratio≤2, irregular, hypoechoic, loss of hilum
3	37/F	Rt level IV	1.5 cm	411.0	Negative	Uptake (−)	L/S ratio≤2, irregular, loss of hilum, cystic change
4	21/F	Rt level IV	1.2 cm	0.3	Negative	Uptake (+)	L/S ratio≤2, irregular, hypoechoic, loss of hilum, calcification

FNAC = fine needle aspiration cytology; LN = lymph node; Tg = thyroglobulin; FDG = ^{18}F-fluorodeoxyglucose; PET/CT = positron emission tomography/computed tomography; L/S ratio = long/short ratio.

found to be 88.2, 100, 100 and 93.8%, respectively (Table 3), and for the BRAF^{V600E} mutation test, they were 64.7, 100, 100 and 83.6%, respectively (Table 4). Every case with a BRAF^{V600E} mutation was diagnosed with LN metastasis, but 12 cases (12.6%) were negative for the BRAF^{V600E} mutation but still diagnosed with LN metastasis. Among these 12 cases, 8 were found to be negative for thyroid cancer nodule in the BRAF^{V600E} test, and 4 were found to be BRAF (-) for LNs and BRAF^{V600E} (+) for thyroid cancer nodules. When these two tests, FNAC and the BRAF^{V600E} mutation test, were implemented together, the diagnoses of only 2 cases (3.1%) were found to be false negative, and the sensitivity and NPV of this new approach of implementing two tests were found to be improved to 94.1 and 96.8%, respectively (Table 5).

DISCUSSION

Although there has been great improvement in the development of imaging modalities and cytological diagnostic techniques, it remains challenging to clearly distinguish between benign and malignant. In addition, there are criteria for the diagnosis of metastatic LNs by USG before surgery, they have not been fully established and its sensitivity and specificity may vary by the criteria. According to a study using node-to-node analysis for LNs matching diagnoses made with USG and results from pathology after surgery, the sensitivity and specificity of USG criteria are as follows: loss of the hyperechoic hilum in a LN (100%, 29%), hyperechoic punctuation (46%, 100%), cystic change (11%, 100%), peripheral vascularization (86%, 82%) and a long to short axis ratio <2 (46%, 64%).(*10*)

Thus far, genetic mutations in various genes including RET, TRK, RAS, and BRAF^{V600E} have been reported in thyroid carcinoma. The BRAF^{V600E} mutation plays a critical role in tumorigenesis, but it is observed in only PTC and PTC-derived anaplastic carcinomas and rarely found in other types of cancers e.g., follicular carcinoma.(*11*) In the

Table 3. The diagnostic accuracy of FNAC for lateral LN metastasis in papillary thyroid carcinoma

	Positive LN metastasis on permanent pathology after surgery (n=34)	Negative LN metastasis on tests before surgery (n=61)	P value
Positive LN metastasis on FNAC (n=30)	30 (100%)	0 (0.0%)	<0.001
Negative LN metastasis on FNAC (n=65)	4 (6.1%)	61 (93.8%)	

FNAC = fine needle aspiration cytology; LN = lymph node.
Sensitivity: 30/34=88.2%, Specificity: 61/61=100%, Positive predictive value: 30/30=100%, Negative predictive value: 61/65=93.8%.

Table 4. The diagnostic accuracy of the BRAF^{V600E} mutation test for lateral LN metastasis in papillary thyroid carcinoma

	Positive LN metastasis on permanent pathology after surgery (n=34)	Negative LN metastasis on tests before surgery (n=61)	P value
Positive LN metastasis on BRAF mutation (n=22)	22 (100%)	0 (0.0%)	<0.001
Negative LN metastasis on BRAF mutation (n=73)	12 (16.4%)	61 (83.6%)	

LN = lymph node.
Sensitivity: 22/34=64.7%, Specificity: 61/61=100%, Positive predictive value: 22/22=100%, Negative predictive value: 61/73=83.6%.

Table 5. The diagnostic accuracy for positives by either of the two tests (BRAF^{V600E} mutation test or FNAC) for lateral LN metastasis

	Positive LN metastasis on permanent pathology after surgery (n=34)	Negative LN metastasis on tests before surgery (n=61)	P value
Positive LN metastasis on FNAC or BRAF (n=32)	32 (100%)	0 (0.0%)	<0.001
Negative LN metastasis on FNAC and BRAF (n=63)	2 (3.1%)	61 (96.8%)	

FNAC = fine needle aspiration cytology; LN = lymph node.
Sensitivity: 32/34=94.1%, Specificity: 61/61=100%, Positive predictive value: 32/32=100%, Negative predictive value: 61/63=96.8%.

republic of Korea, PTC is the most common type of thyroid malignancy (95.6%), and the incurrence rate for $BRAF^{V600E}$ mutations is 63~87%, which is relatively higher than that of other nations (35~65%).(*2*) In general, FNAC is the most widely accepted test for thyroid nodules due to its high accuracy. Despite the high level of sensitivity and specificity for FNAC, it has several limitations, including false-negative and false-positive rates up to 5%. Additionally, there is a report that 10~30% of FNAC results are either non-diagnostic or undetermined, and among them, 20~30% are ultimately diagnosed with a malignancy via pathologic examination.(*12*) Non-diagnostic refers to the absence of follicular cells, limited cellularity, or poor fixation, and indeterminate refers to follicular/Hurthle cell neoplasms or atypical cells of undetermined significance. When the FNAC results are 'indeterminate', it is difficult to choose whether to proceed with treatment. Thus, there are a number of studies on molecular markers such as cytokeratin, galectin-3, RAS, RET, PAX8/PPARγ, and $BRAF^{V600E}$ for overcome the limitations of FNAC and increasing diagnostic accuracy. Among these markers, the $BRAF^{V600E}$ test has received much attention while being known as the most specific and adjunctive diagnostic marker for PTC. There was no report on false positive that $BRAF^{V600E}$ mutation was found in other benign thyroid nodule. According to a study by Salvatore *et al.*(*6*), the $BRAF^{V600E}$ mutation test was found to be helpful for 1 of 4 cases that had an 'insufficient' FNAC result and 3 of 11 cases that had the result of 'indeterminate' for the diagnosis of classic PTC.

When FNAC is implemented alone for a LNs, its sensitivity, specificity, PPV, NPV and accuracy are reported to be approximately 77.3, 98.0, 98.9, 64.0 and 83.3% according to the study of Kim et al, which are not sufficient to be implemented as a single test tool.(*5*) They measured Tg in wash-out fluid from a LN specimen together with FNAC to complement the assay and found that the Tg test increases the sensitivity, and NPV to approximately 95.0 and 87.0%, respectively.(*5*) The Tg test is a useful test, but the absolute cut-off value for Tg has not been established, and it may be affected by the serum Tg. Additionally, when collection of a test sample fails or the quantity of a test sample is not enough, the result could be false-negative, and there is a high chance of erroneous detection of residual thyroid tissue as a LN, causing a false-positive result. Besides, it has been reported that joint administration of FNAC and the $BRAF^{V600E}$ mutation test could increase the sensitivity, specificity, PPV and NPV of diagnosis from 71.4, 95.4, 96.8 and 63.3% to 84.9, 95.4, 97.3 and 76.5%.(*7*) Additionally, it has been reported that the $BRAF^{V600E}$ test could overcome the non-diagnostic and indeterminate limitations of FNAC to a certain extent, and Moon et al. claimed that $BRAF^{V600E}$ mutation analysis is a useful diagnostic tool for suspicious thyroid nodules upon USG.(*8,9,12*) However, there may be a possibility of a false negative of the $BRAF^{V600E}$ mutation test like other tests.

There have been a number of studies of the potential of the $BRAF^{V600E}$ test as a diagnostic tool for thyroid nodules, and they support the notion that the $BRAF^{V600E}$ test is useful as an complementary tool for FNAC and to increase the specificity of the diagnosis.(*13*) However, there have been no studies on the $BRAF^{V600E}$ test as a diagnostic tool for LN metastasis; thus, this report is the first meaningful study. We believe that if the $BRAF^{V600E}$ mutation test can compensate for the limitations of the cell analysis test FNAC, it is possible to suggest a new diagnostic tool for lateral LN metastasis for PTC in a more accurate and specific manner. According to the results of this study, the specificity and PPV are 100% when the $BRAF^{V600E}$ test for LNs detects a mutation and diagnoses metastasis. Additionally, for the new test approach of joint implementation of FNAC and the $BRAF^{V600E}$ test, its sensitivity and NPV are enhanced to 94.1 and 96.8%, respectively, which is higher than that for FNAC and the $BRAF^{V600E}$ test alone. The point is that when FNAC or the Tg test could not lead to a precise result for cystic changes or calcific LNs (indeterminate result), the $BRAF^{V600E}$ test was more useful in reaching a diagnosis. In our study, there were cases actually came to the positive of $BRAF^{V600E}$ test at cystic changed LNs. In cystic LN, recommendation is to aspirate in solid potion rather than cystic portion.

Although BRAF alone had much lower sensitivity and NPV rates. In addition, it was not cost-effective to administer the $BRAF^{V600E}$ test for every suspicious LN due to

its high cost (77 USD). Thus, we selectively recommend using the LN-BRAF^{V600E} test when the patient is found to have suspicious lateral metastatic LNs upon preoperative USG, however, the result is non-diagnostic or indeterminate from FNAC and/or Tg test and want to avoid a frozen biopsy. The frozen biopsy is more accurate, however, requires preoperative tattoo, an extended the incision and more operation times. Additionally, in the Republic of Korea, a patient is responsible for only 5% of the expense of examination if the patient is diagnosed with cancer, and the National Health Service compensates for the remaining expense. Thus, the BRAF^{V600E} test for LNs for PTC patients is not a heavy burden for patients in Korea.

CONCLUSION

In conclusion, the results of this study show that concurrent administration of FNAC and the BRAF^{V600E} test is a supportive diagnostic tool for PTC patients with indeterminate or non-diagnostic suspicious lateral LNs selectively, particularly when they do not have clear diagnostic results from the existing FNAC assay and/or the Tg test. The joint use of these tests may increase the accuracy of LN metastasis diagnosis, which is helpful in terms of determining the type of surgical treatment.

REFERENCES

1. Zuniga S, Sanabria A. Prophylactic central neck dissection in stage N0 papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 2009;135:1087-91.
2. Kim KW. Clinical characteristics of papillary thyroid microcarcinoma. J Korean Thyroid Assoc 2010;3:111-5.
3. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994;97:418-28.
4. Schlumberger MJ. Papillary and follicular thyroid carcinoma. N Engl J Med 1998;338:297-306.
5. Kim MJ, Kim EK, Kim BM, Kwak JY, Lee EJ, Park CS, et al. Thyroglobulin measurement in fine-needle aspirate washouts: the criteria for neck node dissection for patients with thyroid cancer. Clin Endocrinol (Oxf) 2009;70:145-51.
6. Salvatore G, Giannini R, Faviana P, Caleo A, Migliaccio I, Fagin JA, et al. Analysis of BRAF point mutation and RET/PTC rearrangement refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma. J Clin Endocrinol Metab 2004;89:5175-80.
7. Chang H, Lee H, Yoon SO, Kim H, Kim A, Kim BH. BRAF(V600E) mutation analysis of liquid-based preparation-processed fine needle aspiration sample improves the diagnostic rate of papillary thyroid carcinoma. Hum Pathol 2012;43:89-95.
8. Jara SM, Bhatnagar R, Guan H, Gocke CD, Ali SZ, Tufano RP. Utility of BRAF mutation detection in fine-needle aspiration biopsy samples read as "suspicious for papillary thyroid carcinoma". Head Neck 2015;37:1788-93.
9. Agretti P, Niccolai F, Rago T, De Marco G, Molinaro A, Scutari M, et al. BRAF mutation analysis in thyroid nodules with indeterminate cytology: our experience on surgical management of patients with thyroid nodules from an area of borderline iodine deficiency. J Endocrinol Invest 2014;37:1009-14.
10. Leboulleux S, Girard E, Rose M, Travagli JP, Sabbah N, Caillou B, et al. Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. J Clin Endocrinol Metab 2007;92:3590-4.
11. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer 2005;12:245-62.
12. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71.
13. Moon HJ, Kim EK, Chung WY, Choi JR, Yoon JH, Kwak JY. Diagnostic value of BRAF(V600E) mutation analysis of thyroid nodules according to ultrasonographic features and the time of aspiration. Ann Surg Oncol 2011;18:792-9.